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PBPK modeling to support risk assessment of pyrethroid exposure in French pregnant women

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ABSTRACT

Background: Pyrethroids are widely used pesticides and are suspected to affect children's neurodevelopment. The characterization of pyrethroid exposure during critical windows of development, such as fetal development and prenatal life, is essential to ensure a better understanding of pyrethroids potential effects within the concept of Developmental Origins of Health and Disease.

Objective: The aim of this study was to estimate maternal exposure of French pregnant women from biomonitoring data and simulate maternal and fetal internal concentrations of 3 pyrethroids (permethrin, cypermethrin and deltamethrin) using a multi-substance pregnancy-PBPK (physiologically based pharmacokinetics) model. The estimated maternal exposures were compared to newly proposed toxicological reference values (TRV) children specific also called draft child-specific reference value to assess pyrethroid exposure risk during pregnancy *i.e.* during the *in utero* exposure period.

Methods: A pregnancy-PBPK model was developed based on an existing adult pyrethroids model. The maternal exposure to each parent compound of pregnant women of the Elfe (French Longitudinal Study since Childhood) cohort was estimated by reverse dosimetry based on urinary biomonitoring data. To identify permethrin and cypermethrin contribution to their common urinary biomarkers of exposure, an exposure ratio based on biomarkers in hair was tested. Finally, exposure estimates were compared to current and draft child-specific reference values derived from rodent prenatal and postnatal exposure studies.

Results: The main contributor to maternal pyrethroid diet intake is *cis*-permethrin. In blood, total internal concentrations main contributor is deltamethrin. In brain, the major contributors to internal pyrethroid exposure are deltamethrin for fetuses and *cis*-permethrin for mothers. Risk is identified only for permethrin when referring to the draft child-specific reference value. 2.5% of the population exceeded permethrin draft child-specific reference value.

Conclusions: A new reverse dosimetry approach using PBPK model combined with human biomonitoring data in urine and hair was proposed to estimate Elfe pregnant population exposure to a pyrethroids mixture with common metabolites.

Abbreviations: 3-PBA, 3-phenoxybenzoic acid; 4-F-3-PBA, 4-fluoro-3-phenoxybenzoic acid; AOP, adverse outcome pathway; BBB, brain blood barrier; *cis*-DCCA, *cis*-3-(2,2dichlorovinyl)-2,2-dimethylcyclopropane-carboxylic acid; CYP, cypermethrin; DBCA, *cis*-3-(2,2dibromovinyl)-2,2-dimethylcyclopropane-carboxylic acid; DOHaD, Developmental Origins of Health and Diseases; Elfe, French Longitudinal Study since Childhood; Fue, urinary excretion factor; HBM, human biomonitoring; HCT, hematocrit; INED, National Institute of Demographic Studies; Inserm, National Institute of Health and Medical Research; PERM, permethrin; p-PBPK, pregnancy-physiologically based pharmacokinetics; PYR, pyrethroids; SA, sensibility analysis; TK, toxicokinetic; *trans*-DCCA, *trans*-3-(2,2dichlorovinyl)-2,2-dimethylcyclopropane-carboxylic acid; TRV, toxicological reference value.

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1. Introduction

Pyrethroids (PYR) represent a large family of insecticides among the most widely used pesticides in France with various uses (e.g., veterinary, domestic or agricultural) (Saillenfait et al., 2015; Chaperon et al., 2021). Neurotoxicity is a common feature of the pyrethroids toxicity for insects but also for mammals and human. Fetal development is a period of high vulnerability to chemical exposures that can lead to short- and long-term consequences on child’s health, as conceptualized by the Developmental Origins of Health and Disease (DOHaD) (Rager et al., 2020; Charles et al., 2016; Barker, 1998). Recently, early-life pyrethroids exposure was associated to neurodevelopmental disorders in children (Furlong et al.,

2017; Xue et al., 2013; Qi et al., 2022).

In France, prenatal exposure to these widely used pesticides is of particular concern. Pyrethroid levels measured in French pregnant women of the Elfe cohort (Etude Longitudinale Française depuis l’Enfance - French Longitudinal Study since Childhood) were higher than those observed in American studies: CHAMACOS (Center for the Health Assessment of Mothers and Children of Salinas) cohort and NHANES (National Health and Nutrition Examination Survey) (Dereumeaux et al., 2016; Castorina et al., 2010; CDC, 2005). In biomonitoring studies, pyrethroid exposure is often characterized by the measurement of several biomarkers (urinary metabolites) such as: 3-phenoxybenzoic acid (3-PBA), and 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid (DCCA). This high exposure of French population to pyrethroids was already highlighted in the French National Nutrition and Health Survey (ENNS) (Dereumeaux et al., 2016). Due to similar chemical structure and metabolic pathway, these biomarkers are common to various active pyrethroid substances and thus referred to as non-specific pyrethroid metabolites. In France, three main pyrethroids (and their isomers) are suspected to be the major contributors to these biomarkers’ levels: permethrin (cis and trans isomers), cypermethrin (cis and trans isomers) and deltamethrin (Fig. 1) (BNV-D, 2023; ANSES, 2019). These pyrethroids have different toxicity levels with acceptable daily intakes (ADIs) ranging from 0.005 mg/kg bw for cypermethrin to

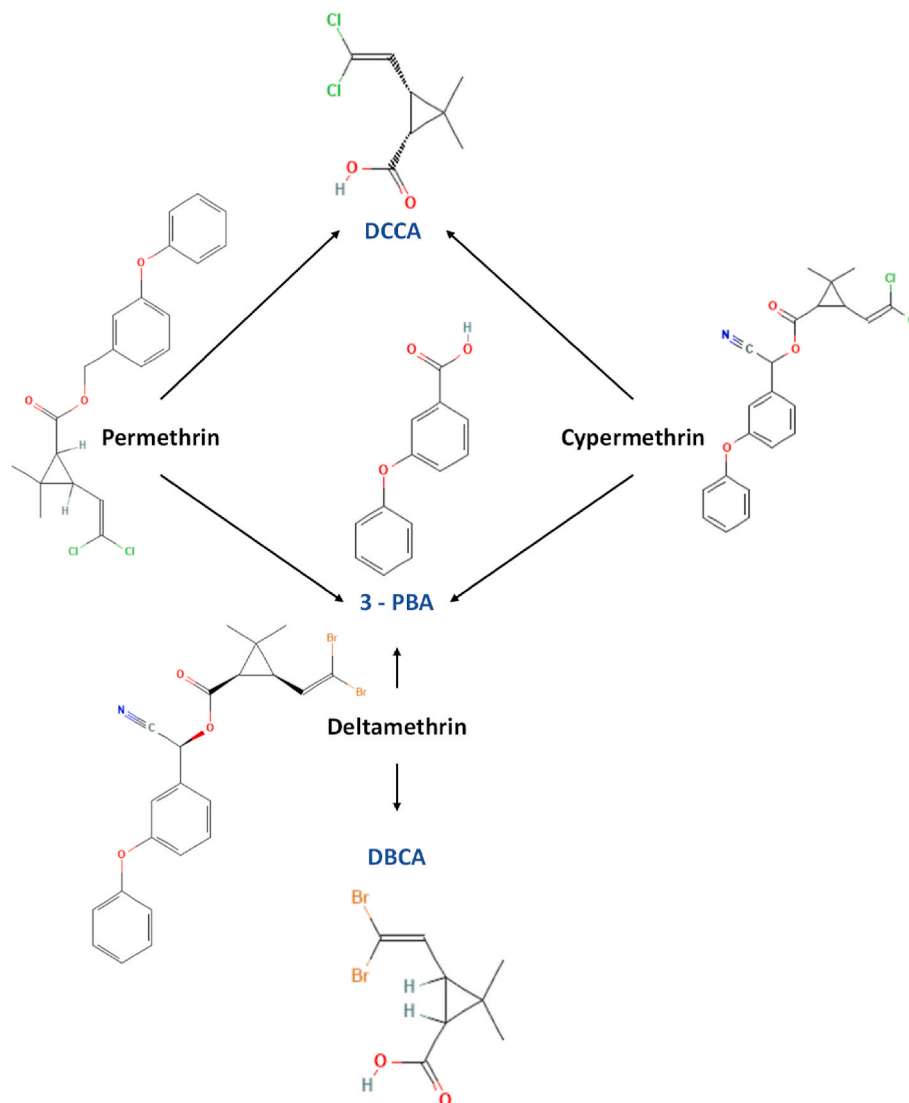


Fig. 1. Pyrethroids of interest and their metabolites (adapted from Ueyama et al. (2010)).

0.05 mg/kg bw for permethrin (Tarazona et al., 2022). Identifying which active substance is the main contributor to the biomarker's levels represent a challenge for risk assessment of pyrethroids.

Human biomonitoring guidance values (HBM-GVs) have previously been proposed for several PYR metabolites (Tarazona et al., 2022), based on existing HBM data from numerous cohorts and ADIs for each of the PYR of interest. Two HBM-GVs screening level for 3-PBA were proposed with conservative hypothesis for adults and children: 4.8 and 3.25 µg/L 3-PBA in urine (Tarazona et al., 2022). When urinary metabolites are common to numerous PYR, such as 3-PBA, the derivation of HBM-GVs is obviously complex. However, tiered biomonitoring equivalents (BE) were calculated from existing BE values and adjusted for the relative potential exposure to each PYR (Aylward et al., 2018). The relative potential exposure was derived from PYR residues found in food samples, as was done in Quindroit et al. (2021) and allows the distinction of each parent compound in the case of common metabolites.

In this work, HBM urinary data of PYR metabolites from the Elfe cohort was used in a reverse dosimetry approach to predict PYR exposure of pregnant population using a p-PBPK (pregnancy-physiologically based pharmacokinetics) model. p-PBPK models simulate the fate of compounds (i.e., the toxicokinetics) in pregnant women using mathematical equations that describe the physiology and anatomy of the mother and fetus, and the absorption, distribution, metabolism, and excretion (ADME) of compounds based on their physicochemical properties (Andersen et al., 1995; Nestorov, 2003; Bois et al., 2022; Brochot et al., 2014; Thépaut et al., 2023). Thus, p-PBPK modeling allows exposure estimation (via reverse dosimetry) and also prediction of internal concentrations (via forward dosimetry) in both mothers and fetuses (Ali Daoud et al., 2023; Brochot et al., 2019). For PYR, PBPK modeling enables to distinguish the parent compound of urinary metabolites, some of which are common to many parent molecules. In our study, a ratio based on hair data was included in the model to consider the relative exposure of permethrin and cypermethrin.

The aim of this study was to assess prenatal exposure to pyrethroids in the French pregnant women of the Elfe cohort (n = 924). The work was divided in three steps i) estimation of PYR exposure in French pregnant women from the Elfe cohort with p-PBPK modeling based on HBM data, ii) prediction of maternal and fetal internal concentrations of PYR and, iii) comparison of exposure estimates with toxicological reference values (TRVs) to assess the potential human health risk of PYR exposure.

2. Methods

2.1. Study population

The Elfe cohort is a French longitudinal cohort that was launched in 2011 by the National Institute of Demographic Studies (INED) and the National Institute of Health and Medical Research (Inserm) described in Vandentorren et al. (2009) and Charles et al. (2011). The analyses of pyrethroids were a part of the perinatal component of French HBM program (Dereumeaux et al., 2016). More than 18,000 children born in France in 2011 were included in the Elfe cohort to characterize factors potentially affecting physical or psychological development, health, and socialization of the child (<http://www.elfe-france.fr>). During the pregnancy of the Elfe children, biological samples (maternal urine and hair at the maternity) were collected in a subgroup of mothers (n = 924). Maternal and fetal individual characteristics data are presented in Table 1.

Four biomarkers of exposure to pyrethroids were measured in the urinary samples: 3-PBA, (cis- DCCA, trans-DCCA and cis-DBCA (cis-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane-carboxylic acid) (Dereumeaux et al., 2016) (Table 2). In total, urinary pyrethroids metabolites data were quantified (i.e. above the limit of quantification LOQ) for 924 women.

In this study, the concentrations in hair are used to set a ratio of

Table 1

Individual characteristics of the pregnant women and their fetuses of the Elfe cohort included in this study.

Characteristics	Mean ± SD
Number of women	924
Age (years)	30 ± 4.8
Maternal weight before pregnancy (kg)	64.7 ± 14.1
Maternal weight at the end of pregnancy (kg)	78 ± 14
Gestational age (weeks)	39.3 ± 1.3
Placental weight (g) (n = 179)	581.5 ± 129.5
Newborn weight at birth (kg)	3.4 ± 0.4

exposure between permethrin and cypermethrin. Only the women for which urinary concentrations and both compounds were quantified in their hair sample (Beranger et al., 2018) were included in to compute the exposure ratio (n = 49, Table 2). Additional data as the dietary habits and the use of phytopharmaceutical products of the Elfe pregnant women collected via questionnaires was used to develop a multivariate model to predict the exposure ratio for the women without hair data and is reported in Supplementary material 1.

2.2. p-PBPK model

The p-PBPK model includes a maternal and a fetal sub-model (Fig. 2). The maternal sub-model includes 23 tissue compartments for the parent compounds. The fetal sub-model is connected to the maternal sub-model through the placenta and has 19 fetal compartments. This p-PBPK model was based on the lifetime model developed by Beaudouin et al. (2010) which was applied to pyrethroids by Quindroit et al. (2019) for adults only. In their model, Quindroit et al. (2019) modified the initial model to account for the pyrethroids' kinetics and to include the kinetics of the metabolites. In our study, only the oral route was considered.

In this model, the organ growth for maternal and fetal parts of the model were described as in the lifetime model of Beaudouin et al. (2010). Since the volume of distribution increase during pregnancy, a hemodilution is observed in pregnant women. Hence, the equation describing hematocrit (HCT) was modified as proposed by Dallmann et al. (2017). Maternal cardiac output was corrected with HCT and increased during pregnancy. This increase in cardiac output leads to an increase in blood flow to all organs during pregnancy. Uterine blood flow was described using an equation developed by Dallmann et al. (2017). Concerning fetal blood flows, equations from Abduljalil et al. (2021) were applied in the model.

Compound specific parameters for parent compounds were set using *in vivo* animal data, due to the lack of *in vivo* or *in vitro* human data. For permethrin, the tissue:blood partition coefficients in mother and fetus

Table 2

Concentrations of PYR metabolites in urine (adapted from Dereumeaux et al. (2016)) and parent compounds in hair (adapted from Beranger et al. (2018)).

Biomarkers of exposure	Median concentrations	LOQ	% of detection	Comments
Urinary metabolite concentrations (n = 924) in µg/L				
3-PBA	0.36	0.014	99.8	Common metabolite for permethrin, cypermethrin, deltamethrin
Cis-DCCA	0.16	0.011	99.3	Common metabolite for permethrin, cypermethrin
Trans-DCCA	0.26	0.019	99.7	Common metabolite for permethrin, cypermethrin
DBCA	0.23	0.016	99.6	Specific metabolite of deltamethrin
Hair sample concentrations (n = 49) in pg/mg				
Permethrin	29.3	10	95.0	–
Cypermethrin	1.22	0.4	83.0	–

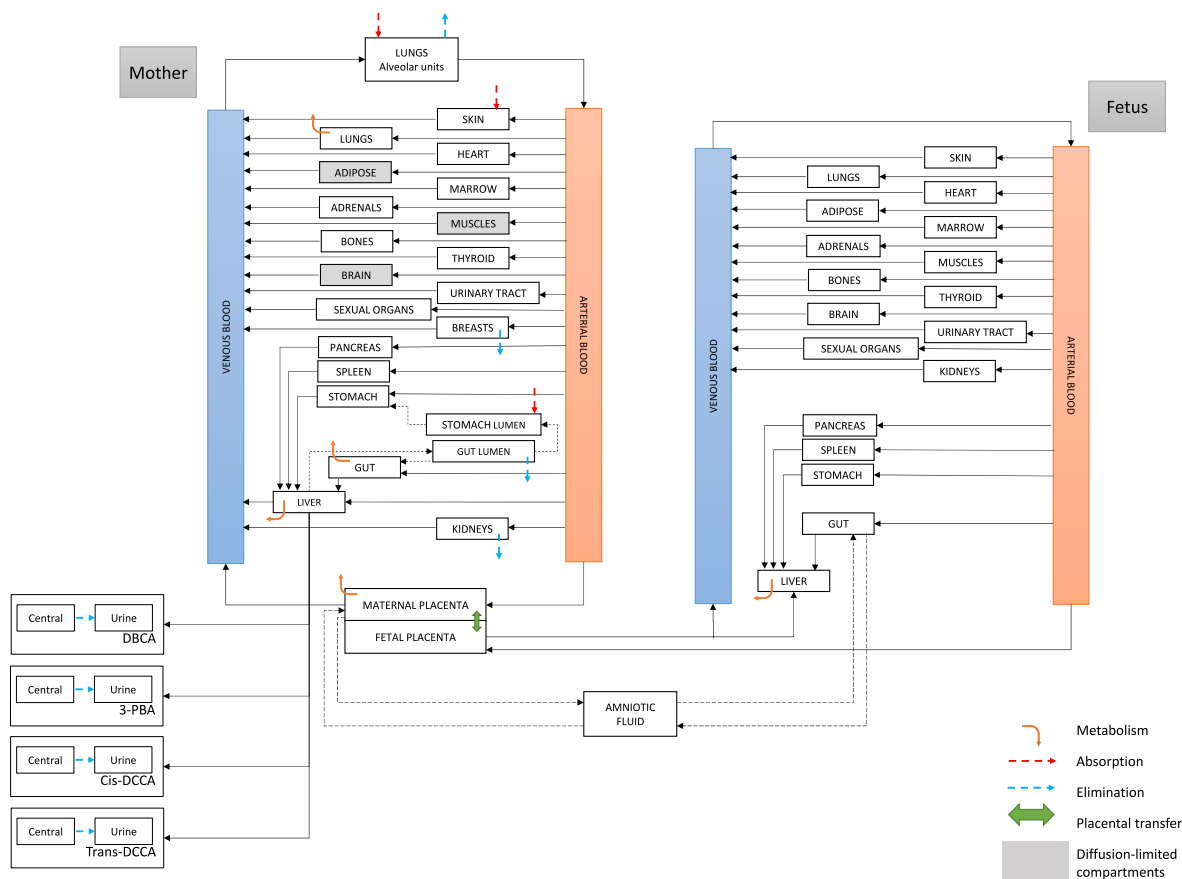


Fig. 2. Schematic representation of the p-PBPK model.

were obtained from a study in which a p-PBPK model was calibrated with toxicokinetic data in pregnant rats (Personne et al., 2021). In mothers, isomer specific values were determined whereas experimental data in fetuses did not allow to estimate isomers values. Because cypermethrin data was lacking, the values of permethrin were used for cypermethrin due to their structural similarities. For deltamethrin, the partition coefficients determined by Mirfazaelian et al. (2006) for adult rats were applied to the mothers (Table B in supplementary material). Placental partition coefficients for both cypermethrin and permethrin were set to the ones proposed by Personne et al. (2021) for permethrin. Deltamethrin placenta:blood partition coefficient was set to the placental partition coefficient of trans-permethrin determined by Personne et al. (2021) due to more similar kinetics compared to cis-permethrin. Placental transfers for the 3 pyrethroids were set as the one calibrated for permethrin (Personne et al., 2021). Data on deltamethrin kinetics in the pregnant population are scarce. However, few *in vivo* studies suggest that DLT accumulates more in juvenile rat brains compared to adults (Sheets et al., 1994; Tornero-Velez et al., 2010). Thus, the fetal partition coefficients for deltamethrin were also set as to those determined for permethrin in Personne et al. (2021) because they describe a higher pyrethroid accumulation in the brain compared to deltamethrin partition coefficient in adults.

For maternal compartments such as the adipose tissue, brain and muscles, the compound's distribution accounts for permeability coefficients to describe the ability of the compound to reach the organ tissue from the blood circulation. These coefficients have been scaled from rat data (Godin et al., 2010; Willemin et al., 2016) to humans (Table B in supplementary material).

Regarding metabolites excretion, the model considers the excretion of 4 metabolites: 3-PBA, cis-DCCA, trans-DCCA and DBCA. Since 3-PBA is a common metabolite to various PYR, a parameter (Rate_Input_PBA)

was taken into account to consider the part of 3-PBA coming from other exposure to PYR, therefore an additional source of 3-PBA was considered into the model.

2.3. Estimation of permethrin/cypermethrin ratio based on hair data

Since permethrin and cypermethrin share the same metabolites (DCCA and 3-PBA), information on the relative exposure to each compound is needed to estimate their individual concentrations. In this study, we proposed to test an individualized approach based on the ratio of the measured permethrin and cypermethrin concentrations in the hair sample from the Elfe cohort (named R_{pm_cyp}). A multivariate model was used on Elfe data to identify which information from the Elfe questionnaires were associated with the permethrin and cypermethrin concentrations in hair. Among the 924 women of interest (for which urinary concentrations were available), 49 women had PYR hair measurements (5.3% of the total). For these women, the log ratio of their own measured concentrations of permethrin and cypermethrin in hair was applied to the PERM/CYP ratio R_{pm_cyp} . Based on these hair concentrations-based R_{pm_cyp} , exposure log ratios were predicted using a 3-way ANOVA for the remaining 875 women without hair concentrations data. Descriptive characteristics of relevant questionnaires data are available in supplementary data (Table A in supplementary material).

2.4. Estimation of the maternal exposure to the PYR mixture

Using the p-PBPK model, maternal diet intake of each PYR was predicted via reverse dosimetry methodology. For each woman in the sub-group of the Elfe cohort ($n = 924$), different parameters such as, the maternal weight before the pregnancy, the maternal age, the placental

weight, the maternal and fetal weight at birth or the gestational age (in weeks) (Table 1) were considered in the model.

In this work, different exposure scenarios were considered to estimate the external exposure: a constant exposure and a scenario with a varying exposure to PYR throughout the day (called “oral intake – 3 meals/day scenario” in the following) starting three days before the delivery. For the constant exposure scenario, a constant urine flow (0.05 L/h (ICRP, 2002)) and a constant void of the bladder was considered, making it an “equilibrium scenario”. For the oral intake – 3 meals/day scenario, three PYR intakes were considered per day, representing the diet intake of PYR. Moreover, 8 voids of the bladder per day were considered (between 8:00am and 10:00pm), in addition to a night/day variation of the urine flow (0.07 L/h and 0.05 L/h) (Aylward et al., 2012; Van Haarst et al., 2004). The oral intake – 3 meals/day scenario considered the last exposure to PYR 9 h before the delivery to represent a mean fasting time during labor (Inde et al., 2018; Shi et al., 2016) and the sampling was considered at delivery.

A Bayesian approach was applied to estimate the diet intake of pyrethroids among the pregnant women of the Elfe cohort. A *prior* uniform distribution was assigned to cypermethrin (for QCYP_ing_rate parameter), deltamethrin (for QDLT_RateIng parameter) and the input of 3-PBA (Rate_Input_PBA parameter) coming from the exposure to other pyrethroids (Table C in supplementary material). For oral intake, repartition of *cis*- and *trans*-isomers was set to 40:60 for permethrin and 42:58 for cypermethrin based on the major pesticide composition found on the market. The likelihood of urinary metabolites concentrations was assumed to follow a lognormal distribution with 15% error. Markov Chain Monte Carlo (MCMC) methods were used to estimate the posterior probability distribution using MCSim (Bois, 2009). Three independent MCMC chains of 10,000 iterations were run, and results are based on the last 6000 iterations. A stable *posterior* distribution was assumed to be reached when the convergence criterion \hat{R} was below 1.2 (Gelman and Rubin, 1996).

2.5. Fetal and maternal internal concentrations predictions using forward dosimetry

Using forward dosimetry approach and the predictions of maternal exposure to PYR, internal concentrations for both the mother and the fetus were predicted. In the same manner as the reverse dosimetry approach, both scenarios were considered to predict internal concentrations.

2.6. Risk assessment

Several agencies and organizations have established toxicological reference values (TRVs) to assess the risk associated with acute, sub-chronic, or chronic oral exposure to cypermethrin, deltamethrin and permethrin. The TRVs are designed to guarantee the absence of effects in the general population. A default uncertainty factor is applied when calculating these values to guarantee the protection of sensitive sub-populations in the absence of specific data. Each of these values was analyzed using Ineris methodology to determine the most relevant value to use in the assessment of health risk (INERIS, 2016). The main elements of method selection are presented in the supplement. In risk assessment, these values are applicable to the general population, including pregnant women, infants and children. An additional analysis aimed at specifically protecting the child sub-population was carried out based on literature data specific to neurodevelopment and led to the proposal of more protective values than those recommended for the general population. The method used to construct these draft reference values follows the recommendations of the Anses (ANSES, 2017), the main elements of which are presented in the supplement. These values were compared with the exposure levels to each pyrethroid estimated for the Elfe pregnant women to assess whether there was health concern for their child.

2.7. Software

The model (DOI: <https://doi.org/10.5281/zenodo.10417319>) was implemented using GNU MCSim v6.2.0 (Bois, 2009). Data analysis and figures were made with R Studio v4.0.2 (R core Team and Computing, 2022).

3. Results

3.1. Comparative exposure to permethrin and cypermethrin

With a multivariate model, several parameters filled in Elfe questionnaires were identified as affecting the permethrin and cypermethrin concentrations in hair: the uses of phytopharmaceutical products (against fleas and ticks or against crawling insects) and the maternal weight before the pregnancy (Table 3). A 3-way ANOVA predicted the PERM/CYP ratio for the women without measured data in hair, based on the 49 PERM/CYP ratio obtained from measured concentrations of PYR in hair (Table 3).

The exposure to cypermethrin and permethrin was compared based on the ratio of their concentrations in maternal hair. For each of the 49 women with hair samples, their individual PERM/CYP ratios were calculated with a mean value of 1.43 ± 0.59 (Table 3), with a minimal ratio of 0.14 and a maximal ratio of 2.41. Most of the 49 women had a pre-pregnancy BDW inferior or equal to 75 kg and did not report use of pesticides (44.9%) or did report using products against fleas and ticks (26.5%). For the remaining 875 pregnant women including in our study with no hair samples, the PERM/CYP hair ratio was imputed using the multivariate model (Table 3). The mean value of the PERM/CYP ratio for all the women ($n = 924$) is 1.58 ± 0.23 . The maximum and minimum PERM/CYP ratio are 2.41 and 0.14 respectively.

Most of the women (51.3%) belong to the category of mothers with a pre pregnancy bodyweight inferior to 75 kg and no reported use of pesticides. These women have a PERM/CYP ratio predicted to 1.57 (Table 3). The PERM/CYP ratio ($Rpm_cyp = 1.68$) is increased (i.e., a higher permethrin exposure than cypermethrin one) when mothers have reported using products against fleas and ticks. Mothers reporting use of products against crawling insects have a lower predicted PERM/CYP ratio (0.58 or 1.36 depending on maternal weight before pregnancy).

To evaluate the PERM/CYP ratio used in this work, a comparable PERM/CYP ratio was calculated based on oral exposure estimates by

Table 3

Predicted ratio and number of women for which different Rpm_cyp was applied. BDW: bodyweight, FT products: fleas and ticks products, CI products: crawling insects products. (95% confidence interval in brackets).

Different cases	Percentage of the population (n = 875)	Predicted PERM/CYP ratio
Use of CI products and FT products		
Pre pregnancy BDW >75 kg	0.5%	1.71 [1.17; 2.25]
Pre pregnancy BDW ≤75 kg	2%	1.63 [1.28; 1.97]
Use of CI products only		
Pre pregnancy BDW >75 kg	1.1%	0.58 [-0.63; 1.78]
Pre pregnancy BDW ≤75 kg	7.3%	1.36 [0.90; 1.81]
Use of FT products only		
Pre pregnancy BDW >75 kg	4.8%	1.68 [1.20; 2.06]
Pre pregnancy BDW ≤75 kg	17.3%	1.68 [1.53; 1.83]
No use of pesticides reported		
Pre pregnancy BDW >75 kg	10.4%	1.68 [0.99; 2.38]
Pre pregnancy BDW ≤75 kg	51.3%	1.57 [1.37; 1.77]

Quindroit et al. (2019). In their study, a mean total oral intake of $0.020 \pm 0.100 \mu\text{g}/\text{kg}/\text{day}$ and $0.015 \pm 0.075 \mu\text{g}/\text{kg}/\text{day}$ were calculated for permethrin and cypermethrin respectively, resulting in a PERM/CYP ratio of 1.3 ± 6.9 , which is in the same range as our individualized ratio based on hair measurements of 1.43 ± 0.59 .

3.2. Maternal exposure estimates by reverse dosimetry

External exposure to pyrethroids was estimated for the equilibrium and dietary exposure scenarios from the urinary concentrations of the PYR metabolites for all the pregnant women using the reverse dosimetry approach. The estimated urinary metabolite concentrations were in good agreement with the observations, as shown in predicted vs observed metabolite concentrations figures in supplementary material 4. Overall, the model reproduced the measured urinary metabolite data well: 100% of the predictions fall within the 5-fold interval, with at least 90% of the predictions falling within the 2-fold interval. Concerning the specific metabolite of deltamethrin, DBCA, 100% of the predictions are in the 2-fold interval. The predictions of common metabolites 3-PBA, cis-DCCA and trans-DCCA were, respectively, 91%, 96% and 98% falling within the 2-fold interval. No 3-PBA predictions are below the identity line.

Figs. 3 and 4 present the boxplots of the predicted maternal exposure to PYR for both scenarios. For the dietary exposure scenario, the estimates of the PYR exposure for each meal were normalized over one day to be compared with the estimates obtained with the equilibrium

scenario. Calculations for the normalization are presented in supplementary material 5.

Descriptive statistics of the pyrethroids' exposure estimated using the equilibrium scenario are presented in supplementary material 6. The median exposure of the women from the Elfe cohort is $14.3 \text{ ng}/\text{kg bw}/\text{day}$ for cypermethrin, $22.6 \text{ ng}/\text{kg bw}/\text{day}$ for permethrin and $13.2 \text{ ng}/\text{kg bw}/\text{day}$ for deltamethrin. The differences between the mean and the median of the predicted exposure (Table D in supplementary material 6) demonstrate the variability of the exposure in the population. The maximum estimated external exposures were 1729, 2714 and 274 $\text{ng}/\text{kg bw}/\text{day}$ for cypermethrin, permethrin and deltamethrin, respectively. A similar table describing the predicted exposure for each PYR with the oral intake – 3 meals/day scenario is available in the supplementary material (Table E in supplementary material 6).

The median cumulative PYR intake is $55 \text{ ng}/\text{kg bw}/\text{day}$ with permethrin, deltamethrin and cypermethrin contributing to it at 45%, 26% and 29% respectively. The maximum cumulative PYR intake is $4472 \text{ ng}/\text{kg bw}/\text{day}$, with a contribution of permethrin, deltamethrin and cypermethrin of 60%, 1% and 39% respectively to the total PYR intake.

In both scenarios, the estimated daily exposures for the three pyrethroids are within the same range. However, on average, the cumulative PYR exposure estimated with the equilibrium scenario represents 60% of the cumulative PYR exposure estimated with the oral intake – 3 meals/day scenario.

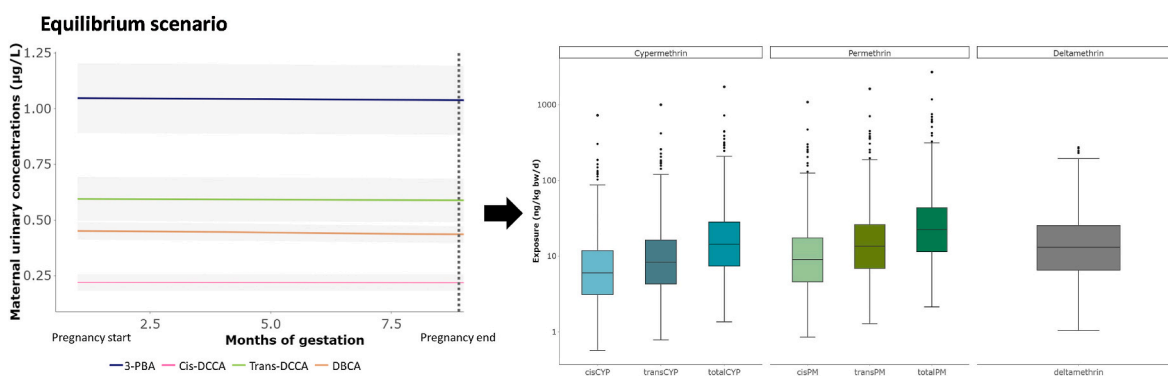


Fig. 3. Boxplot representing the exposure predictions of Elfe women ($n = 924$) using the equilibrium scenario. On the left are represented the urine concentration of metabolites prior sampling. Here are represented the 3-days before the urine sampling which occurred at birth. 95% confidence interval from the urinary metabolite concentrations are represented in the shaded grey area. cisCYP: cis-cypermethrin, transCYP: trans-cypermethrin, totalCYP: sum of cis- and trans-cypermethrin, cisPM: cis-permethrin, transPM: trans-permethrin, totalPM: sum of cis- and trans-permethrin.

Oral intake – 3 meals/day scenario

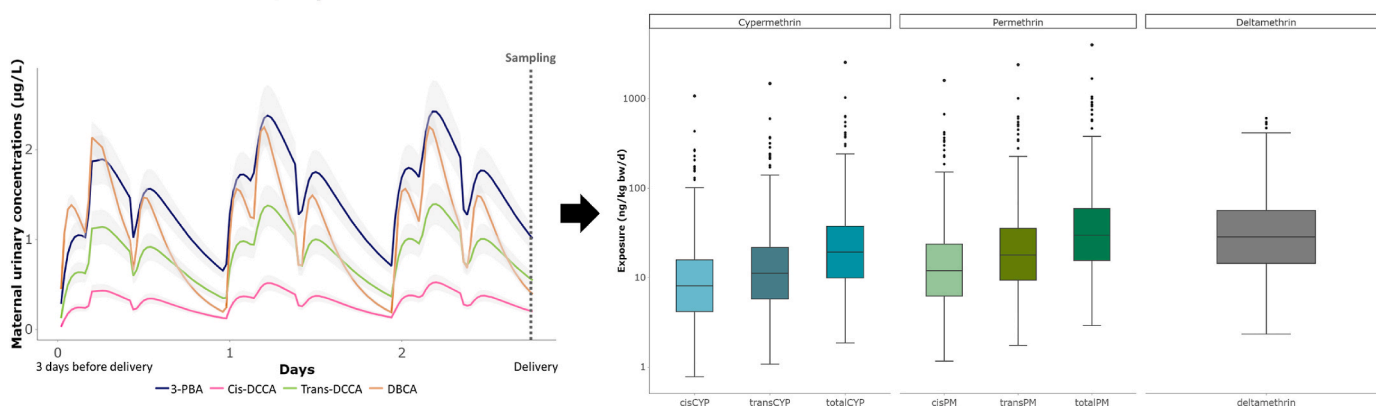


Fig. 4. Boxplot representing the exposure predictions of Elfe women ($n = 924$) using the oral intake – 3 meals/day scenario. On the left are represented the urine concentration of metabolites prior sampling. Here are represented the 3-days before the urine sampling which occurred at birth. 95% confidence interval from the urinary metabolite concentrations are represented in the shaded grey area. cisCYP: cis-cypermethrin, transCYP: trans-cypermethrin, totalCYP: sum of cis- and trans-cypermethrin, cisPM: cis-permethrin, transPM: trans-permethrin, totalPM: sum of cis- and trans-permethrin.

The parameter Rate_Input_PBA was considered in the model to take into account for the fraction of 3-PBA excreted coming from exposure to PYR other than permethrin, cypermethrin and deltamethrin. The contribution of Rate_Input_PBA to the global share of 3-PBA excreted was below 0.001%, indicating that most of the measured 3-PBA can be explained by the estimated exposure to permethrin, deltamethrin and cypermethrin. The contribution of other PYR not included in our study is then estimated to be negligible.

3.3. Internal concentrations by forward dosimetry

The estimated external exposure to PYR was used to predict internal maternal and fetal concentrations using a forward dosimetry approach. Both exposure scenarios were performed. The contribution of each PYR to the total PYR internal exposure (*i.e.*, concentration) in the maternal and fetal blood and brain was calculated and is shown in Fig. 5. In maternal and fetal blood, the major contributor is deltamethrin. In brain, the main contributor to the global PYR exposure differs between mothers and fetuses, with *cis*-permethrin and deltamethrin respectively. This difference can be explained by the different maternal and fetal partition coefficients in addition to the brain permeability coefficient considered for the mother (Table B in supplementary material).

The contributions of PYR to both maternal and fetal blood exposure and brain are presented in Fig. 6 for two women, demonstrating the variability of maternal and fetal internal concentrations within the Elfe population. Each woman has specific contributors to global PYR exposure, depending on the urinary metabolite excretion: for some women, deltamethrin is the major contributor to the global exposure to PYR (93%), whereas for others, deltamethrin contribution to the PYR exposure is minor.

Median PYR concentrations in maternal and fetal brain and blood throughout pregnancy are presented in Fig. 7 for the oral intake – 3 meals/day scenario and in supplementary material 7 for the equilibrium scenario. Regarding the equilibrium scenario, the highest median concentration in maternal and fetal blood is for deltamethrin, reaching respectively 109.6 pg/L and 61.7 pg/L. In brain, the maximum median PYR concentrations are 206.1 pg/L for the mother (*cis*-permethrin) and 247.5 pg/L for the fetus (deltamethrin). The median brain PYR concentrations are up to about 4 times higher than in blood for both the mother and her fetus. Maternal blood concentrations are up to 1.8 times

higher compared to the fetus.

Concerning the oral intake – 3 meals/day scenario, the maximum median PYR concentration in blood for both mother and fetus is deltamethrin, reaching respectively 2000 pg/L and 250 pg/L. In the brain, deltamethrin has the maximum predicted concentration for both mother (2200 pg/L) and fetus (1000 pg/L). Like the equilibrium scenario, the fetal brain concentrations are at least 4 times higher than in blood. For the mother, 2.5 times higher brain concentrations are observed for the *cis* isomers pyrethroids compared to the blood concentrations. However, little accumulation is observed for the *trans*-isomers of pyrethroids and deltamethrin in the brain.

Regarding the equilibrium scenario, a steady state is reached in the brain and in the blood in both mother and fetus within a few hours after the beginning of exposure. However, for the oral intake – 3 meals/day scenario, the maximum concentration is reached after a 3-day exposure for the mother and a 5-day exposure for the fetus, for both blood and brain compartments and remains the same maximum concentration from then on. The predicted daily intake determined with urinary metabolites considering mass balance equation at steady state (Apel et al., 2020) with proportional urinary excretion factor (*F_{ue}*) (Tarazona et al., 2022) are very similar to those predicted with the *p*-PBPK model in the same conditions. For the comparison, the *p*-PBPK model was split for each pyrethroid and for each metabolite (Supplementary material F).

3.4. Risk assessment

Existing TRVs for chronic oral exposure were analyzed for the three pyrethroids and led to the recommendation of the Anses from Efsa value of 5 µg/kg bw/d for cypermethrin (Efsa, 2018; Anses, 2021), the WHO value of 10 µg kg bw⁻¹.d⁻¹ for deltamethrin (WHO JMPR, 2001) and the Health Canada value of 20 µg/kg bw/d for permethrin (Health Canada, 2017)(Table 4). While these values account for neurotoxic effects, they only consider neurodevelopmental effects by adding an uncertainty factor. Thus, although there are limitations related in particular to the exposure durations investigated, values specific to children for sub-chronic exposures were calculated from recent data in the literature of 5 µg/kg bw/d for cypermethrin, 0.8 µg/kg bw/d for deltamethrin and 0.17 µg/kg bw/d for permethrin, respectively (Pitzer et al., 2019; Saito et al., 2019; Gomez-Gimenez et al., 2017) (Table 4). Both studies of Saito et al. (2019) and Gomez-Gimenez et al. (2017) have been conducted on

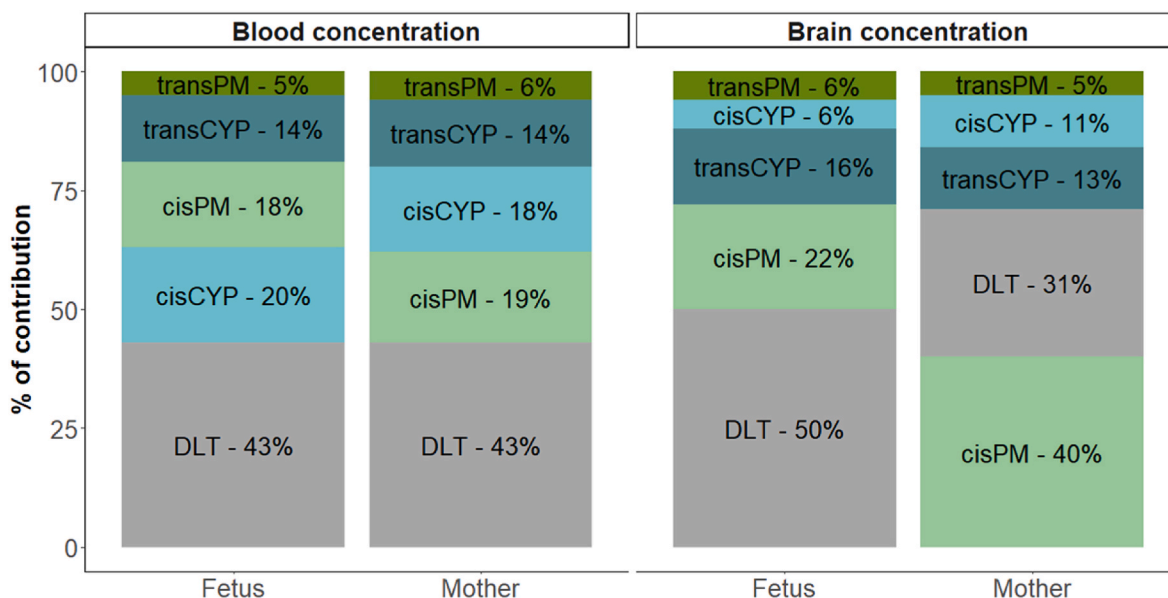


Fig. 5. Mean contributions to internal concentrations in pyrethroids for both blood and brain for mothers and fetuses from the Elfe cohort. DLT: deltamethrin, cisPM: cis-permethrin, transPM: trans-permethrin, cisCYP: cis-cypermethrin, transCYP: trans-cypermethrin.

Table 4
Table of recommended existing TRVs and draft specific values for children.

		Cypermethrin	Deltamethrin	Permethrin
Recommended TRV for chronic exposure of the general population	Value (µg/kg bw/d)	5	10	20
	Organization	EFSA (2018), ANSES (2021)	WHO JMPR (2001)	HEALTH CANADA (2017)
Draft child-specific reference value for sub-chronic exposure	Value (µg/kg bw/d)	5	0.8	0.17
	Source study	Gomez-Gimenez et al. (2017)	Pitzer et al. (2019)	Saito et al. (2019)

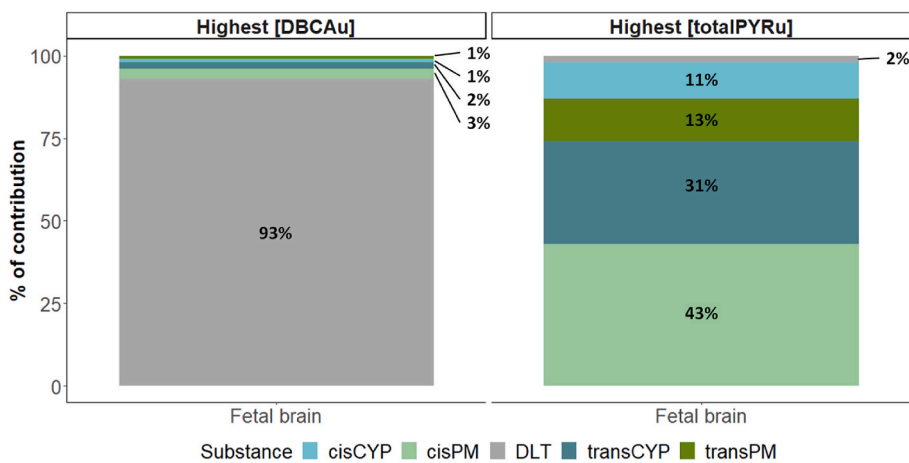
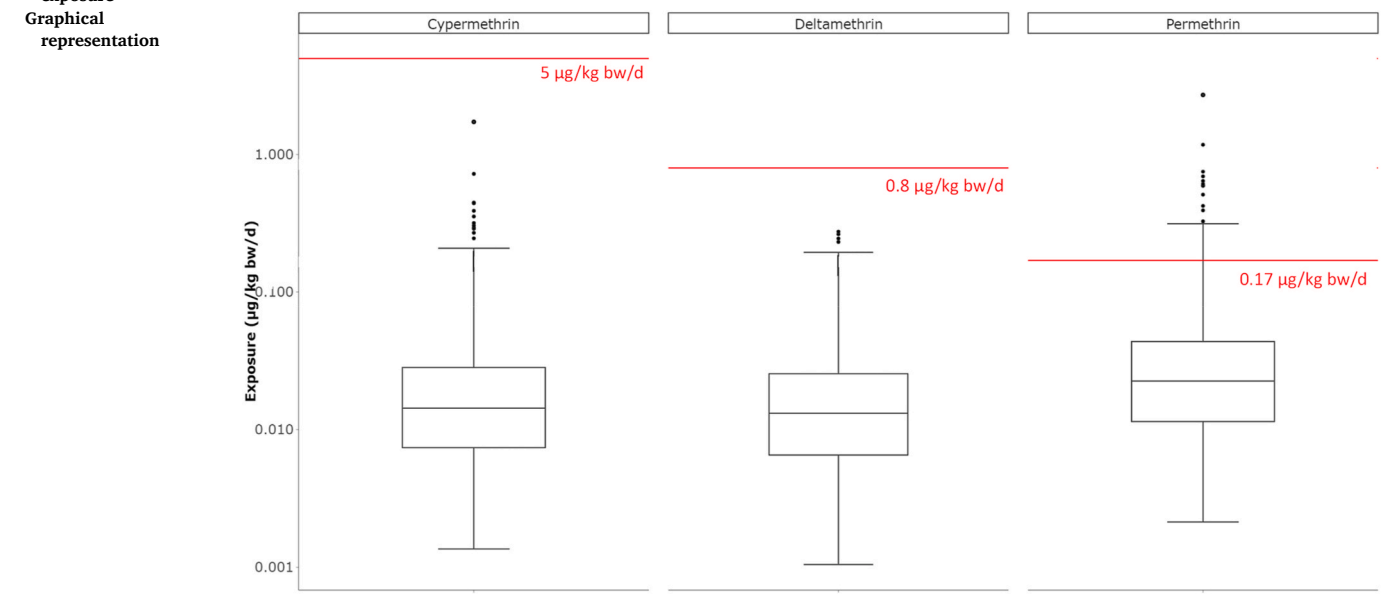


Fig. 6. Contributions of each pyrethroids to the total pyrethroid fetal brain concentrations for two women from the Elfe cohort. On the left, the woman with the highest urinary DBCAu concentration measured in urine (5.4 µg/L) and on the right the woman with the highest overall (3-PBA: 36 µg/L and trans-DCCA: 38 µg/L) urinary metabolite concentrations. DLT: deltamethrin, cisPM: cis-permethrin, transPM: trans-permethrin, cisCYP: cis-cypermethrin, transCYP: trans-cypermethrin.

gestating rodents that were exposed to permethrin or cypermethrin *in utero* and during juvenile development. The exposure period covered in these studies is roughly equivalent to that of pregnancy in women. These draft child-specific reference value show that lower values would provide better protection for this population (detailed calculations in [Supplementary material 9](#)). Given the available data, it was only possible to propose TRVs for sub-chronic exposure. As a precaution, an additional factor (often 3) is usually added to take account for extrapolation from sub-chronic to chronic exposure.

When the external exposure values calculated by our model with

both scenarios are compared with the TRV for chronic oral exposure for the general population, no effect is expected. The 95th percentile of our population exposures to permethrin, cypermethrin and deltamethrin are below the draft child-specific reference value which can be interpreted as no increased risks. However, if the draft child-specific reference values are used for the comparison, 2.5% of the population are exposed to permethrin above the 0.17 µg/kg bw/d, which could represent a health concern (Table 4). For the other two pyrethroids (cypermethrin and deltamethrin), the external values calculated are below the draft child-specific reference values.

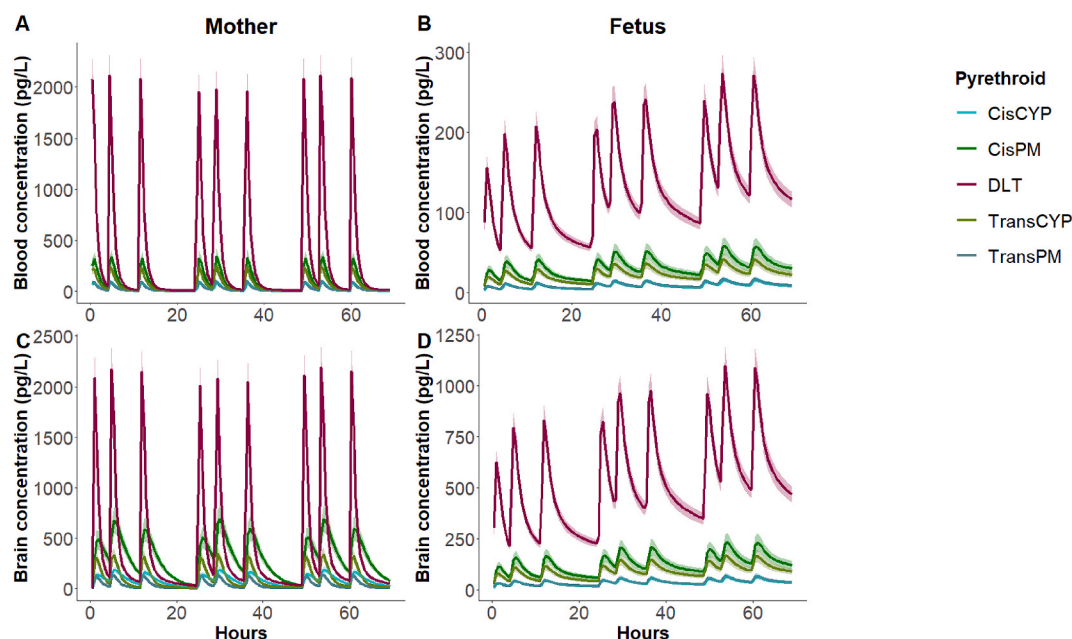


Fig. 7. Median internal concentrations in the blood and the brain for mothers and fetuses. 95% confidence interval are represented in the colored shaded area. DLT: deltamethrin, cisPM: cis-permethrin, transPM: trans-permethrin, cisCYP: cis-cypermethrin, transCYP: trans-cypermethrin.

4. Discussion

PBPK modeling is a useful tool in risk assessment of multiple substances to improve hazard characterization (EFSA Scientific Committee, 2019). Indeed, development of PBPK models regarding mixtures of such pesticides could inform on the contributions of each parent compounds to the urinary metabolite concentrations retrieved from HBM studies. Our work was focused on three molecules (permethrin, cypermethrin including their cis and trans isomers and deltamethrin) and their major urinary metabolites. To this end, an existing adult PBPK model (Quindroit et al., 2019) was extended to the pregnant population to integrate the pregnancy and the development of the fetoplacental unit. In the present model, the fetus is described with 19 compartments. The structure of the whole-life model was kept in this work for further investigations. The metabolites are described as a single one-compartment model with two key parameters, the fractions formed from parent compounds and the urinary elimination rates. For chemical specific parameters, due to the lack of *in vitro* or human data, animal data were used when available. However, the amount and quality of data available was different between the three compounds. For permethrin, partition coefficients have been established for each isomer in the mother and the fetus by calibration using p-PBPK modeling in pregnant rats (Personne et al., 2021) and were used in our study. For deltamethrin partition coefficients have been determined in a PBPK model in adult rats (Mirfazaelian et al., 2006) and were kept unchanged in our model for the mother. For cypermethrin isomers, due to the lack of data, specific partition coefficients values for each isomer in the mother were set to the same values as those of *cis* and *trans*-permethrin isomers in rats (Personne et al., 2021). For fetal partition coefficients, usually unpredicted fetal partition coefficients are set as the mother' (Thépaut et al., 2023). In this case, because of difference in toxicokinetics (TK) between adults and juveniles, permethrin fetal partition coefficients were set for deltamethrin and cypermethrin. Parameterization is a decisive step in PBPK modeling and can affect the output of the PBPK model, when data are sparse or unavailable, which is particularly the case of cypermethrin. The current p-PBPK model was validated for the pregnant population, however no available data for fetuses allowed the validation of fetal predictions. The good agreement of PYR metabolites predictions with the observations rely on the metabolites fraction determined in

Quindroit et al. (2019). However, new experimental data on fetal TK and placental transfer such as the study of Personne et al. (2021) for each PYR may help to reduce parameters uncertainty link to gestation. Several studies have highlighted the association of cognitive impairment in children with pyrethroid metabolite concentrations in pregnant women urinary samples at urinary metabolite levels similar to those measured in the Elfe cohort (Fluegge et al., 2016; Qi et al., 2022; Watkins et al., 2016), supporting the need for complementary studies and most importantly *in utero* data following pyrethroid exposure.

As permethrin and cypermethrin share the same common metabolites 3-PBA, cis-DCCA and trans-DCCA (Chaperon et al., 2021; Angerer and Ritter, 1997), an exposure ratio was defined to differentiate the proportion of metabolites formed from external exposure to each parent compound. Previous studies such as in Quindroit et al. (2019) used consumption and contamination data from food control surveys. However, these indirect data are not specific to our population. In the case of ENNS exposure predictions in Quindroit et al. (2019), the PERM/CYP ratio was calculated from each individual food consumption and diet contamination to pyrethroids (ANSES, 2011). In the adult model of Quindroit et al. (2019), contamination data from the French food control survey Total Diet Study 2 (ANSES, 2011) were used in combination with consumption of food for each subject to provide this information (ratio permethrin/cypermethrin). However, neither cypermethrin nor deltamethrin were detected in any samples and permethrin was only detected in 0.14% of the food samples. Due to the poor sensitivity of the analytical method used in the Total Diet Study 2, there were some uncertainties in the calculated exposures.

As hair analysis were included in the Elfe cohort study, we calculated an individual ratio of measured concentrations of permethrin and cypermethrin in hair of women for which both hair and urine samples were available. However, only 49 subjects fulfilled this criterion. In women for whom only urine samples were available, concentration in hair was extrapolated from the data of the Elfe questionnaires by means of a multivariate model to identify several categories. A different ratio was calculated for each category and was then mean value of each category was imputed for women without hair results depending on the category. The PERM/CYP ratio predicted with ANOVA (Table 3) increased when parents reported using pesticides against fleas and ticks and decreased when they reported using products against crawling

insects. Our results are supported by the applications of both pyrethroids. For example permethrin is usually used against fleas, ticks, scabies, and head lice while cypermethrin is used to control crawling insects such as cockroaches (Ravula and Yenugu, 2021). The PERM/CYP ratio in the hair was used as a surrogate for the PERM/CYP exposure ratio to which the pregnant women were exposed. Hair represents a rather recent matrix considered in HBM studies.

This study proposes to investigate a new strategy to better characterize exposure to PYRs using hair concentrations in PBPK modelling applied to exposure estimation. Biomarker hair concentrations are relevant for the assessment of frequent exposures because of the potential accumulation of chemicals in hair which unlike other matrices such as urine or blood, makes it possible to retrace an individual exposure on a long term (Appenzeller and Tsatsakis, 2012; Appenzeller et al., 2017).

However, this study highlights that internal kinetics of the parent compounds in humans are lacking for model validation and also for investigating the relationship between blood and hair concentrations. Here, deltamethrin was identified as a major contributor to blood concentrations of PYR, while deltamethrin concentrations in hair are below those measured for permethrin and cypermethrin (Beranger et al., 2018). The majority of study data and models focus on the urinary excretion of PYR metabolites (*i.e.* metabolism and excretion) (Mallick et al., 2020; Ratelle et al., 2015; Quindroit et al., 2019) and do not investigate differences in absorption between parent pyrethroids, which is suspected by previous studies on bioaccessibility (Shi et al., 2017). Kinetics of parent compounds in blood remain a key tool to better understand accumulation of biomarkers in hair. Using hair measurements is an appealing approach to get exposure information and could be considered in further biomonitoring studies. This approach has been applied to pyrethroids in this study but can be extended to other pesticide compounds that also share common metabolites, such as neonicotinoids or organophosphates (Sudakin and Stone, 2011; Wrobel et al., 2022).

One of the main objectives of our work was to reconstruct plausible exposure of the French pregnant women from the Elfe Cohort (2011) to each pyrethroid by reverse dosimetry using PBPK model accounting for cumulative exposure to the three substances. In our work, we considered two scenarios, a constant exposure scenario to allow appropriate comparison with previous studies as well as a more realistic scenario with a daily dietary exposure to visualize the intra-day variability of biomarkers. Our exposure predictions for constant scenario (75th percentile exposure estimates: permethrin: 0.04 $\mu\text{g}/\text{kg bw}/\text{d}$, cypermethrin: 0.03 $\mu\text{g}/\text{kg bw}/\text{d}$ and deltamethrin: 0.03 $\mu\text{g}/\text{kg bw}/\text{d}$) remain in the same range as the ones predicted by Quindroit et al. (2019) for the general French population based on biomonitoring data of the ENNS cohort in 2006–2007 (75th percentile exposure estimates: permethrin: 0.05 $\mu\text{g}/\text{kg bw}/\text{d}$, cypermethrin: 0.10 $\mu\text{g}/\text{kg bw}/\text{d}$ and deltamethrin: 0.12 $\mu\text{g}/\text{kg bw}/\text{d}$) (Table I in supplementary material). Compared to other studies, such as Darney et al. (2018) who were interested in estimating the total exposure of the French adult population to permethrin only, our estimates are encompassed between their “lower” (75th percentile exposure estimate: 0.010 $\mu\text{g}/\text{kg bw}/\text{d}$) and “intermediate” (75th percentile exposure estimate: 0.52 $\mu\text{g}/\text{kg bw}/\text{d}$) scenarios. Aylward et al. (2017) estimated that the American population exposure to PYR via diet was mostly driven by permethrin, followed by cypermethrin and deltamethrin exposures, such as the pregnant women from the Elfe cohort (75th percentile of exposure estimates: permethrin: 1.80 $\mu\text{g}/\text{kg bw}/\text{d}$, cypermethrin: 0.61 $\mu\text{g}/\text{kg bw}/\text{d}$ and deltamethrin: 0.002 $\mu\text{g}/\text{kg bw}/\text{d}$). The equilibrium scenario (*i.e.* a constant exposure to PYR) was usually considered in similar work such as in the study of (Quindroit et al., 2021), due to frequent exposure to such substances. It also facilitates the comparison of exposure estimates with the ones calculated with tiered approach which also consider a steady state conditions for exposure to non-persistent substances like pyrethroids (Tarazona et al., 2022) and is more interpretable in terms of daily intake rates (Aylward et al., 2017).

The approach used by Tarazona et al. (2022) in Tier 1 considering steady state condition and in a conservative each pyrethroid predicted daily intake of permethrin, cypermethrin and deltamethrin within the same range as our model predictions’ at steady state (Table F in supplementary material). More complex scenarios such as the oral intake – 3 meals/day scenario allow to represent concentration variability within a day for PYR. In addition, the internal concentrations for example in blood in such realistic scenarios are higher than those predicted by a simpler constant exposure scenario (*i.e.* the equilibrium one). Our study has some limitations to consider individual realistic scenarios. In fact, realistic modeling of specific scenarios may require specific cohort data on pesticide use and most importantly timeline of potential exposure. In addition, the time of the last use of phytopharmaceutical products or the time of the last food intake compared to the time of the urine sample are crucial for the biomarker’s levels, as it has been demonstrated with the diet intake scenario. Moreover, urinary concentrations were only available at a single time point. Due to the relatively short half-life of the PYR metabolites, repeated measures would have been useful to take into account for within-person variability. In fact, multiple sampling, over a day or over a longer period of time, would have represented the daily variability of urinary metabolite excretion (Aylward et al., 2012). Throughout pregnancy, multiple sampling periods could illustrate the seasonal variations in pyrethroid exposure (Tang et al., 2021).

The second objective of our work was to use our individual exposure estimates to predict the internal dose of each compound, especially in the fetal brain. Pregnancy-PBPK model allows prediction of fetal internal concentrations of xenobiotics, for which measured data are unavailable (Atoyebi et al., 2019; Codaccioni and Brochot, 2020; Thépaut et al., 2023). Unlike the major contributor to external pyrethroids exposure, permethrin, the overall major contributor to internal pyrethroid exposure is deltamethrin. Deltamethrin was identified as the major contributor to the blood concentration of pyrethroids in both mothers and fetuses. However, the major contributors to pyrethroids concentrations in the brain differed between mothers and fetuses, being *cis*-permethrin and deltamethrin, respectively. This result is consistent with previous studies showing that the accumulation of deltamethrin in brain is more important in the brain in juveniles than in adults (Sheets et al., 1994; Tornero-Velez et al., 2010). In this model, the placental transfer is assumed to be simple diffusion. Yet, data on active placental transfer remain sparse, and the transfer of PYR across the placenta could involve active transporters, which could redefine fetal exposure to PYR. However, permethrin has been described as poorly able to permeate the BBB (Brain-Blood Barrier), which suggest that it is not a substrate for active transport and could only be subject to passive permeation through the BBB (Deepika et al., 2022). Placental transfer was previously estimated for permethrin in pregnant rat (Personne et al., 2021). Given that data on placental transfer remain sparse and the structural similarities between the molecules, the same placental transfer was used for the three pyrethroids of interest here. Placental transfers data generation from *in vivo*, *ex vivo* or *in vitro* experiments will be necessary for a better characterization of transplacental exchanges, especially for cypermethrin and deltamethrin.

Finally, this study assessed the risks to the fetuses of the Elfe population by comparing the estimated maternal exposures with the TRVs. Usually, TRVs are established for the general population, including pregnant women, newborns, and children. Our analysis of existing TRVs for cypermethrin, deltamethrin and permethrin showed that, while these values are well constructed for neurotoxic effects, considering specific sub-populations means adding an additional uncertainty factor. These results are consistent with those of Tarazona et al. (2022). Recent studies on neurodevelopmental effects in rodents (Pitzer et al., 2019; Saito et al., 2019) have allowed us to propose draft child-specific reference value for sub-chronic exposure durations in line with these studies.

Measuring exposure in pregnant women enables us to estimate the exposure of the fetus to xenobiotics. Thus, maternal exposure to

pyrethroids has been compared to draft child-specific reference values because it has been shown that fetal exposure to xenobiotics can lead to adverse effects at lower concentrations (Falck et al., 2015). No pregnant women were exposed at level above the drafted child-specific TRV for sub-chronic exposure for either permethrin, cypermethrin or deltamethrin when the 95th percentile is considered. However, measurement of the exposure of pregnant women in the Elfe cohort to pyrethroids showed that 2.5% of these Elfe women were exposed to permethrin at levels above the TRV determined for the child sub-population, which raises concern.

The draft child-specific reference value for permethrin was calculated from a study conducted on rats using repeated exposure before mating, during gestation and during the postnatal period up to the age of 28 days, i.e. an overall exposure of approximately 2 months, showed that exposure to 500 µg/kg bw/d induced a delay in learning, as measured by the conditioned fear test (Saito et al., 2019). This delay is associated with an increase in immature neurons and a decrease in astrocytes in the hippocampus and dentate gyrus (Saito et al., 2019). These results are consistent with data relating to other pyrethroids (Shafer et al., 2005) and highlight the action of pyrethroids on the hippocampus and neurogenesis. Thus, the measurement of other pyrethroids in all the mothers in the Elfe cohort analyzed in our study raises concerns that their effects may be potentiated. The hypothesis of cumulative effects in adult rats has been assessed for acute exposure to 11 pyrethroids on motor function using an additivity of relative potencies approach (Wolansky et al., 2009), but cannot be extrapolated to longer exposure times. However, these effects have not been found in humans for chronic exposure: it has been shown that there is no neurotoxic risk (motor and sensory effects) in adults in a French cohort for aggregate exposure to 4 pyrethroids (Vanacker et al., 2020). As the effects on neurodevelopment were identified at lower doses than in adults, it would appear useful to continue the work for exposure durations of around 3 months (for the consideration of chronic exposure) and for mixtures of pyrethroids.

5. Conclusion

In conclusion, we have developed a pregnancy PBPK model that allows the use of pyrethroid biomonitoring data for health risk assessment. A novel approach was described here, with the integration of hair concentration data as part of reverse dosimetry, as a complement to urinary metabolite concentrations. The p-PBPK model was used to estimate the distribution of exposure of pregnant women to each PYR, taking into account common urinary metabolites. These exposures were compared with existing TRVs and draft child-specific reference values. 2.5% of the Elfe population was identified as being above the draft child-specific reference values with no effect for permethrin, raising concerns about the safety levels of exposure to pyrethroids. The collection of complementary data on pyrethroid distribution in the mother and fetus, in addition to the studies conducted to derive TRVs, should be considered for a better understanding of pyrethroid toxicity during pregnancy.

CRedit authorship contribution statement

Elisa Thépaut: Writing – original draft, Visualization, Validation, Software, Methodology, Formal analysis, Data curation. **Michèle Bisson:** Writing – original draft, Formal analysis. **Céline Brochot:** Writing – original draft, Supervision, Software. **Stéphane Personne:** Writing – review & editing. **Brice M.R. Appenzeller:** Writing – review & editing, Resources, Methodology. **Cécile Zaros:** Methodology. **Karen Chardon:** Writing – review & editing, Supervision, Conceptualization. **Florence Zeman:** Writing – original draft, Validation, Supervision, Software, Project administration, Methodology, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The link to the code is shared in the methods.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envres.2024.118606>.

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